# The effects of RAMPs upon cell signalling

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## Abstract

G protein-coupled receptors (GPCRs) play a vital role in signal transduction. It is now clear that numerous other molecules within the cell and at the cell surface interact with GPCRs to modulate their signalling properties. Receptor activity modifying proteins (RAMPs) are a group of single transmembrane domain proteins which have been predominantly demonstrated to interact with Family B GPCRs, but interactions with Family A and C receptors have recently begun to emerge. These interactions can influence cell surface expression, ligand binding preferences and G protein-coupling, thus modulating GPCR signal transduction. There is still a great deal of research to be conducted into the effects of RAMPs on GPCR signalling; their effects upon Family B GPCRs are still not fully documented, in addition to their potential interactions with Family A and C GPCRs. New interactions could have a significant impact on the development of therapeutics

## Keywords

Receptor activity modifying protein, G protein-coupled receptor, signalling, trafficking, coupling.

## Abbreviations

AM, adrenomedullin; AMY, amylin; CaSR, calcium-sensing receptor, CGRP, calcitonin gene-related peptide; CHO, chinese hamster ovary; CLR, calcitonin receptor-like receptor; CT, calcitonin; CTR, calcitonin receptor; CRF, corticotrophin releasing factor; ECD, extracellular domain; ECL, extracellular loops; GCGR, glucagon receptor; GLP, glucagon-like peptide; GLP, GLP2R, glucagon-like peptide receptor 2; GPCR, G protein-coupled receptor; GPR30, G protein coupled estrogen receptor 1; GRKs, G protein-coupled receptor kinases; h, human; HEK, human embryonic kidney; m, mouse; NHERF-1, Na+/H+ exchanger regulatory factor-1; PTX, pertussis toxin; PTH, parathyroid hormone; PTHR, parathyroid hormone receptor; PTHrP, parathyroid hormone related peptide; r, rat; RAMP, receptor activity modifying protein; s, salmon; VPAC, vasoactive intestinal peptide.

## 1. Introduction

In order to communicate and respond to their surrounding environment, cells utilise a vast array of signalling molecules ranging from neurotransmitters,

photons of light, lipids and hormones. Signals from many of these molecules are transduced by G protein-coupled receptors (GPCRs) which comprise the largest family of membrane proteins, with more than 800 of these seven transmembrane domain receptors now identified in the human genome[1]. As such, these receptors play a crucial role in mediating most physiological responses and are implicated in many disease states, making them valuable targets for drug development.

In the classical model, upon receptor activation, GPCRs undergo a conformational change and activate an associated heterotrimeric G protein. GDP is exchanged for GTP on the Gα subunit, which dissociates from the βγ subunit. These liberated subunits then activate downstream effector molecules such as adenylyl cyclase and phospholipase C, resulting in stimulation or inhibition of an intricate web of signalling pathways within the cell to control processes including transcription, translation and metabolism [2, 3](Fig. 1). There are 16 known Gα subunits, 5β and 12γ in humans, with the potential of hundreds of combinations[4]. In addition, there are thought to be G protein-independent signalling pathways activated by GPCRs[2] such as through β arrestins[5] and G protein-coupled receptor kinases (GRKs)[6].

GPCRs are much more complex than first envisioned; they were initially thought to behave like switches, with an inactive state and no signalling, or an active state initiating a signalling cascade. It is now clear that GPCRs occupy numerous conformations, which are associated with the activation of a range of signalling pathways. These conformations are stabilised by ligands, therefore certain agonists bias the receptor for a particular pathway or combination of pathways in comparison to another[3]. Complicating this system further, many GPCRs have been shown to interact with additional components[7]. Allosteric modulators bind to the receptors at a different location to the orthosteric ligand binding site. This further influences the pharmacology by altering orthosteric ligand affinity or efficacy, and in some cases may themselves act as allosteric agonists or antagonists[8, 9].

One such group of proteins that can have a significant impact upon GPCR location, ligand binding and signalling are the receptor activity modifying proteins (RAMPs), which were first identified through research into possible CGRP (calcitonin gene-related peptide) receptors. One of the candidates, the then orphan Family B GPCR calcitonin receptor-like receptor (CLR), was difficult to study and responses to CGRP only appeared to occur in HEK293T cells and not others such as COS7 cells lines[10]. This information suggested the requirement of another component for a functional receptor, which was present in HEKs. The elusive component was discovered 1998, when McLatchie *et al* injected Xenopus oocytes with the cDNA of SK-N-MC cells, which contain endogenous CGRP receptors. They identified a population of cells with larger responses to CGRP and isolated the cDNA of a 148 amino acid single-pass membrane protein, which they named RAMP1[11]. Upon co-expression of CLR with RAMP1 in cells that did not contain endogenous CGRP receptors, a response to CGRP was observed

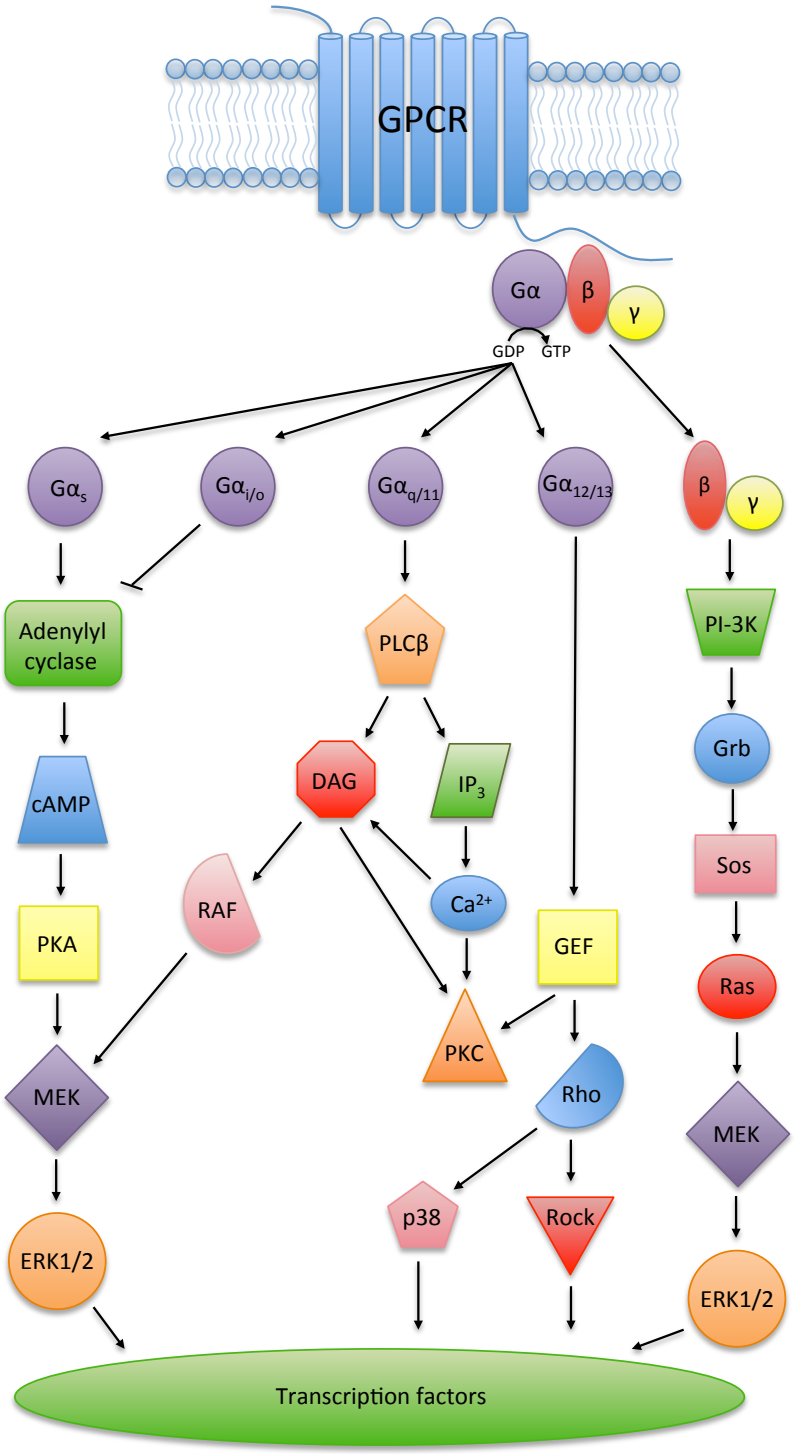


**Figure 1.** Signalling pathways of GPCRs.

equivalent to that seen in SK-N-MCs. Further investigation demonstrated that RAMP1 was required to transport CLR to the cell surface in order to form a functional receptor able to become bound and activated by CGRP[11]. Database searches identified two RAMP-like proteins named RAMP2 and RAMP3 with 31% homology to one another. RAMP2 and RAMP3 were found to form the adrenomedullin 1 and 2 receptors (AM1R, AM2R) together with CLR[11, 12]. The RAMPs by themselves, like CLR, show only poor cell surface expression; however the RAMP/CLR heterodimers are efficiently trafficked to the outside of the cell.

The interactions of the RAMPs with CLR and calcitonin receptor (CTR) are now well studied, providing us with better insight into the role of these accessory proteins[13]. It is now known that RAMPs can interact with some GPCRs to alter the pharmacology of the receptors by allosterically affecting the structure, altering ligand specificity and pharmacology, and in trafficking certain receptors. Several Family B receptors have now been shown to interact with the RAMPs, in addition to emerging interactions with GPCRs from Family A and C (summarised in Table 1). The consequences of these interactions in many cases are still unclear. Here we discuss research that has been conducted to investigate the role of RAMPs upon GPCR signalling; these findings are highlighted in Table 2. Other aspects of RAMPs have been recently reviewed elsewhere[14].

## 2. RAMP interactions with Family B GPCRs

### 2.1 CLR

The role of RAMPs in translocating CLR to the cell surface have been described above; it should further be noted that CLR by itself appears to be unable to bind with appreciable affinity any of the endogenous peptide ligands within the CGRP/calcitonin family. Two recent studies have cast some light on how RAMPs can influence peptide binding to CLR. Crystal structures of the extracellular domain (ECD) of CLR in combination with either the ECD of RAMP1 and a CGRP analogue or RAMP2 and an adrenomedullin (AM) fragment show that the RAMPs interact with the C-terminal residue of the peptide (F37-amide for CGRP, Y52-amide for AM). For CGRP, F37 contacts W84 of RAMP1. (Fig. 2a). In RAMP2, the equivalent residue, F111 cannot make the necessary contact but instead there is an interaction with E101and Y52 of AM (Fig. 2b). In RAMP1, the equivalent of E101, W74, fails to contact CGRP.There are no further direct contacts between either peptide and the RAMPs. Instead the peptides have turn structures, not seen in other peptide ligands for family B GPCRs which contact CLR. There is evidence for some small but potentially significant RAMP-dependant shifts in the conformation of the contact residues on CLR, suggesting that the RAMPs act in part by allostery[15].

The RAMPs also seem to exert an effect on the extracellular loops (ECLs) of CLR. This has been investigated by mutagenesis; for each RAMP a different set of residues within the ECLs appear to be important. On the basis of molecular modelling, it has been suggested that RAMP-induced conformational changes in ECL3 may be particularly important[16].